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Formulation and characterization of P^H sensitive hydrogel of teteracycline for the treatment of *Helicobacter pylori* induced peptic ulcer

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Research Article

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Abstract – pH responsive hydrogels are found to deliver the drugs at a controlled rate to the delivery site at the controlled release. The following research is aimed to formulate and design formulations of ph responsive hydrogels comprising of chitosan and poly acrylic acids. The formulations were designed based on 3^2 factorial design . The formulations were subjected to various evaluations such as invitro drug release and mucoadhesion test. When the concentration of PAA decreases as a result the drug release slightly increases. It also results in greater mucoadhesion and maximum swelling at 0.1 M HCL. The optimized formulation showed that Higuchi model.
Keywords – hydrogel, teteracycline, *Helicobacter pylori,* peptic ulcer

Introduction

Controlled drug delivery system provide an alternative approach to regulate the bioavailability of therapeutic agents in controlled drug delivery systems, an active therapeutic is incorporated into a polymeric network structure. In such a way the drug is released from the material in a pre defined manner. Depending on the drug delivery formulation and the application, the drug release time may be anywhere from a few hours to a month to several years [1] a variety of synthetic and natural polymers have been studied as drug carriers, and drug delivery system have capitalized on their wide-ranging hydrophobic and hydrophilic components and their polymer-polymer, polymer-drug, polymer-solvent or polymer-physiological medium interactions.

The physical and chemical properties drug deliveries specifically regret their permeability environmental response, surface functionality and biodegradability and biorecognition sites to produce intelligent drug delivery. Biodegradable and biocompatible hydrogels have been designed using natural polymers that are susceptible to enzymatic degradation are using synthetic polymers that possess hydrolysable moieties. Of these hydrogels using natural polymers chitosan has received a great deal of attention due to their well documented biocompatibility, low toxicity and degradability by human enzymes.

Hydrogels are of special interest in control release applications because of their soft tissue biocompatibility and ease with which drugs are dispersed in matrix and high degree of control achieved by selecting the physical and chemical properties of the polymer network. In case of diseases involving peptic ulcers it has been demonstrated that *Helicobacter pylori* is one of the major causative agent this bacterium releases enzyme urease, which converts urea into ammonia and bicarbonate which aids in neutralizing the acidic medium and allow the bacteria to colonize in gastric mucosa [2].

The main objective of the present work is to develop p^H response drug delivery for tetracycline using Chitosan and poly acrylic acid hydrogels used in the treatment of gastric ulcer caused by *H. Pylori*. Chitosan and poly acrylic acid hydrogels were prepared with different composition of co-polymers.

Helicobacter pylori is a gram negative bacillus uniquely adapted to survival in the hostile environment in the stomach. It attaches to the surface epithelium beneath the mucus, has high urease activity produces ammonia which maintains a neutral microenvironment around the bacteria, and promotes back diffusion of H^+ ions. It has been found as a commensally in 20-70% normal individuals, and is now accepted as an individuals, and is now accepted as an important contributor to the causation of chronic gastritis, dyspepsia, peptic ulcer, gastric lymphoma and gastric carcinoma.[3,4] Tetracycline was selected as a model drug. Various attempts have been done in the controlled release of antibiotic drug delivery using Chitosan and poly acrylic acid polmers [5,6,7,8].

Antimicrobials that have been found clinically effective against *H. pylori* are: amoxicillin, clarithromycin, tetracycline and metronidazole, tinidazole.

The aim of this work is to formulate a Controlled release of Hydrogel using chitosan and poly acrylic acid polymers, as a trial method. Tetracycline was selected as a model drug. Various attempts have been done in the controlled release of antibiotic drug delivery using Chitosan and poly acrylic acid polmers. Here the plasma level is well controlled and the drug release in the stomach, so the action of drug is varies and the pH is not correctly determined in the stomach. The twice a day formulation is to increase the plasma level concentration and the dose intervals is increased, so the adverse effect is less and the patient compliance is high.

Materials and methods

Tetracycline was obtained from Gift sample from Micro Labs, Hosur. Chitosan , Poly Acrylic Acid (PAA), Glacial acetic acid and Hydrochloric acid from S.D fine chem. Ltd., Mumbai, and other chemicals obtained are of analytical grade.

Standard Calibration Curve of 0.1M HCl

100 mg of Tetracycline was accurately weighed and dissolved in 100 ml of 0.1M hydrochloric acid. 1 ml of stock solution was pipette out into 100 ml of volumetric flask and volume was adjusted with 0.1M hydrochloric acid to get a concentration of 10µg/ml. The stock solution was diluted with 0.1M hydrochloric acid to get concentrations from 0.5 to 3µg/ml. The absorbance of this diluted solution was measured at 274 nm. The absorbance was plotted against concentration.

Drug –Polymer Interaction Study

FT-IR Studies

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 4000 and 400 cm^{-1}. The spectra obtained for Tetracycline and physical mixtures of Tetracycline with chitosan and poly acrylic acid were compared to check compatibility of drug with polymers.

Differential scanning calorimetry

Thermograms of the samples were obtained in Differential scanning calorimeter (Pyris 6 DSC, software Pyris manager, Perkin-Elmer Schweiz AG, Hunenberg, Switzerland). Samples of 8.730 mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10°C/min over a temperature range of 40 to 230°C.

Preparation of Hydrogels

The composition of different formulations used in the study containing 500 mg of Tetracycline in each case is shown in table 1 and 2. They are formulated using the 3^2 factorial designs.

Table 1: Designing of the formulation

Sl. No	*Interaction*		*Chitosan (mg)*	*PAA (mg)*	*Code*
	A	**B**			
1	-1	-1	150	150	F1
2	-1	0	150	100	F2
3	-1	+1	150	75	F3
4	0	-1	200	150	F4
5	0	0	200	100	F5
6	0	+1	200	75	F6
7	+1	-1	225	150	F7
8	+1	0	225	100	F8
9	+1	+1	225	75	F9

Table 2 : Composition of F1-F9 formulations in (mg)

Sl. No	*Formulation Code*	*Tetracycline (mg)*	*Chitosan (mg)*	*Poly acrylic acid (PAA)*
1	F1	500	150	150
2	F2	500	150	100
3	F3	500	150	75
4	F4	500	200	150
5	F5	500	200	100
6	F6	500	200	75
7	F7	500	225	150
8	F8	500	225	100
9	F9	500	225	75

a) Chitosan solution was prepared in 1%v/v acetic acid under stirring.
b) Chitosan and PAA were used in different ratios to prepare nine formulations F1-F9.
c) 500mg of tetracycline was added to chitosan solution and stirred for 15 minutes.
d) PAA dissolved in distilled water was added to chitosan solution gradually under stirring.
e) The solution obtained was immediately poured into petridish and poly ionic complexes were kept overnight at room temperature.
f) The hydrogels obtained were dried for 24 hrs under vacuum.

The dried hydrogels were crushed and passed through sieve #60 and #85. Those hydrogel particles passed through sieve #60 but retained on #85 were used for further studies.

Characterisation of hydrogel

***Invitro* Drug release study**

The ability of prepared hydrogels to retard drug release in the physiological environment of the Gastro intestinal tract(GIT) was assessed by conducting drug release studies in USP basket apparatus and stirring speed of 100 rpm at 37±0.5^0 C in 900 ml of dissolution medium (i.e 0.1N HCL, and pH 7.4) for 8 hours. At predetermined intervals, samples were withdrawn and

spectrophotometrically assayed for drug concentration at 274 nm.

***Invitro* Drug Release Kinetics**

In controlled drug delivery of hydrogels, first these particles absorbs the surrounding water and swell then the drug content is slowly releasd from the swellable layers and diffuses in to the surrounding medium and the drug is release from the hydrogels. In this work hydrogels were prepare by physical mixing of the drug with polymers in various ratios . The following mathematical models were evaluated considering the dissolun datas.These datas are fitted into the kinetic equations using MS-EXCEL having add on kintetics software.

Zero-order model

The drug release from the dosage from follows a 'steadt-state-release' running at a constant rate

$$M_t/M_a = kt$$

Where M_t amount of drug released at time t.
M_a the maximal amount of the released drug at infinite time
K is the rate constant of drug release

First-order model

The activity with in the reservoir is assumed to decline exponentially and the release rate is proportional to the residual activity.

$$M_t/M_a = 1- \exp(-kt)$$

Higuchi square root time model

The most widely used model to describe drug release from matrices, derived from Higuchi for a planar matrix, however it is applicable for systems of different shapes too;

$$M_t/M_a = kt½$$

Korsemeyer-peppas equation

This model describes the mechanism of release

$$M_t/M_a = kt^n$$

Where, n is the release exponent and it is used to characterize different release mechanism.

Swelling property

The swelling property of hydrogels were carried out by placing a known amount (30 mg) of hydrogels in 20 ml of 0.1 M HCL and at every one hour interval, the hydrogels were filtered and excess surface liquid was removed by blotting and their weights were recorded .

The swelling process was characterized by

%Swelling = $(W_s-W_d)X\ 100/W_d$

Where, W_s = weight of the swollen hydrogel

W_d = weight of dried hydrogel

Percentage of Mucoadhesive Study

Mucoadhesive study was performed by taking a known weight(50mg) and placed on the pieces of intestinal mucosa (2x2 cm) of albino rat were placed on to glass slides (3x1 inch) with cyanoacrylate glue. About 50 mg were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of USP tablet disintegrating test apparatus and tissue specimen was given a slow regular up and down movement in the test fluid at 37±0.5°C. At every 1hinterval the samples were withdrawn and the readings are taken and calculated the percentage of mucadation.

Percentage of mucoadhsation = xer4zxs

$$\frac{\text{Initial amount} - \text{cumulative drug release}}{\text{Initial amount}} \times 100$$

Acute Oral Toxicity Studies

The procedure was followed by using OECD guidelines (organization of economic corporation and development) 423(acute toxic class method).

The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances. This procedure results in the use of a specified number of animals while allowing for acceptable data-based scientific conclusion.

The method used defined doses (2000mg/kg body weight) and results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for classification of chemical which cause acute toxicity. Three male mice 30 gm were used for the study, the starting dose level of prepared Hydrogel was selected 2000mg / kg / b.w /p.o. the drug was administered orally to mice which were fasted over night with water before administration of the drug. Body weights of the rats before and after treatment were noted. Any changes in skin and eyes and mucous membrane and also respiratory, circulatory, autonomic, CNS, somato motor activity, behavior pattern were observed. The onset of toxicity and signs of toxicity if any were also noted for a period of 14 days.

Scanning Electron Microscopy (SEM)

SEM studies were carried out on hydrogel samples. The samples were coated with gold-palladium and magnified under 23000X and 24000X to study the surface morphology of hydrogel micro particles.

Stability Studies

To assess the drug and formulation stability, stability studies were done according to ICH and WHO guidelines. Selected best formulation was sealed in aluminium packaging coated inside with polyethylene, was kept in the humidity chamber maintained at 45 °C and 75 % relative humidity for 2 months. At the end of studies, samples were analyzed for the drug content, in vitro dissolution study and other physicochemical parameters.

Result and discussion

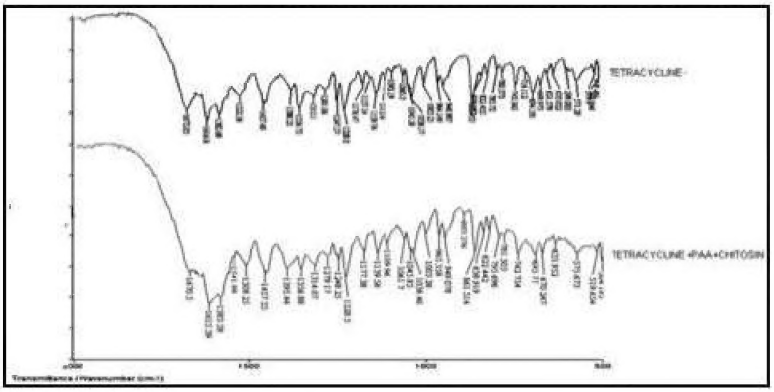
Compatibility studies

FT-IR studies

FTIR spectra of drug and polymers were recorded separately and in combination. The results are represented in figure 1.IR-spectra of pure tetracycline showed major bands at 1670.5 c=0 for acetic acid , 3364.41forAr-OH acetic acid,2850 C-CH3 metylgroups, c=c at1457.5was observed similar bands were observed when tetracycline along with polymers mixed. That results shows that there is no considerable change was observed in bands of tetracycline

and indicates that there is no interaction between drug and polymers.

Figure 1: Comparative study of I.R spectra for determination of drug polymer interaction

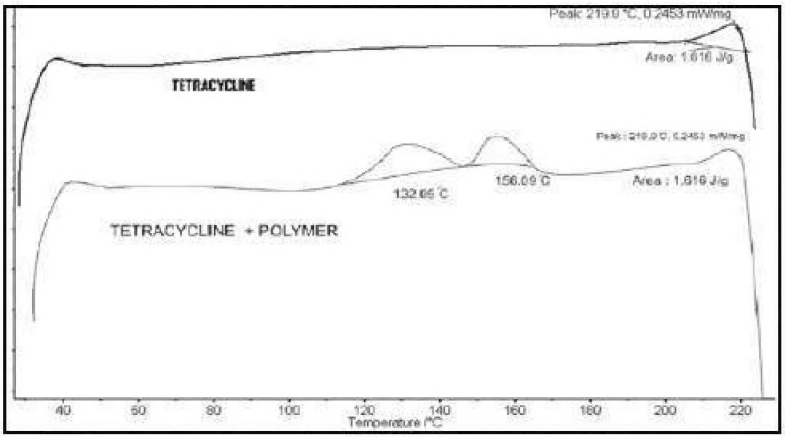


DSC Analysis

DSC thermogram of tetracycline,polymers, and its physical mixture with polymers are presented in below. The DSC thermogram of Figure 2 shows an exothermic peak at 219°c corresponding to the melting temperature of tetracycline.which conform that the drug was tetracycline. The DSC thermogram of shows that an exothermic peaks at 132.05°c and 156.09°c.which indicates that the polymers was chitosan and poly acrylic acid.

The below characterstic peaks appeared in the thermograms indicating that no interaction between the drug and polymers.

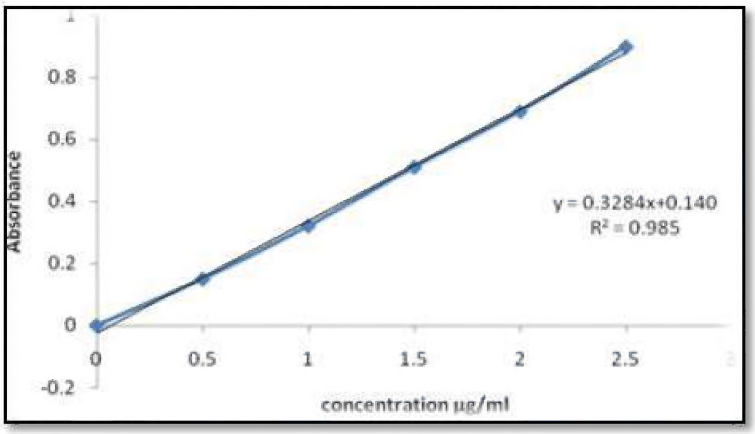
Figure 2: The DSC thermogram



Calibration Curve

From the U.V absorbance study it was concluded from the liner regression analysis that the R^2 value of 0.998. The equation for the resultant calibration curve in 0.1 M HClwas y=0.3284x+0.140 as from the line shown in figure 3.

Figure 3: Standard curve of Tetracycline in 0.1 M hydrochloric acid



Affect of polymer concentrations on invitro drug release

The drug release of the nine formulation showed that F6 with highest drug release as in table 4 and figure 5, later as the drug release was correlated with the polymer by using factorial design When the concentration of PAA decreases as a result the drug release slightly increases .F1-F3 with the constant amount of chitosan 150mg, F4-F6 with the constant amount of chitosan (225) and F7-F9 with the constant amount of chitosan (200) given in table 6. From these results it is evident that hydrogel with lower content of PAA, F6,1:0.33, lower concentration of PAA showed greater mucoadhesion and maximum swelling at 0.1 M HCL. It was also observed that drug release greatly depend on the PAA concentration .The maximum release obtained at 8th hr was 94% with lowest concentration of PAA. The optimized formulation was subjected to rate kinetics study ,out of the various study models followed like Zero order, First order, Higuchi and Koresmeyer-Peppas as shown in figure 4. The R^2 value of the line equation was determined. The study reviled that Higuchi model showed a better R^2 value. Thus further study showed that the drug release follows a Fickian release mechanism, where n=0.50,k=0.19. From the statically ANOVA data table 3 it can be understand that each polymer shows its characteristic feature and influence one another.

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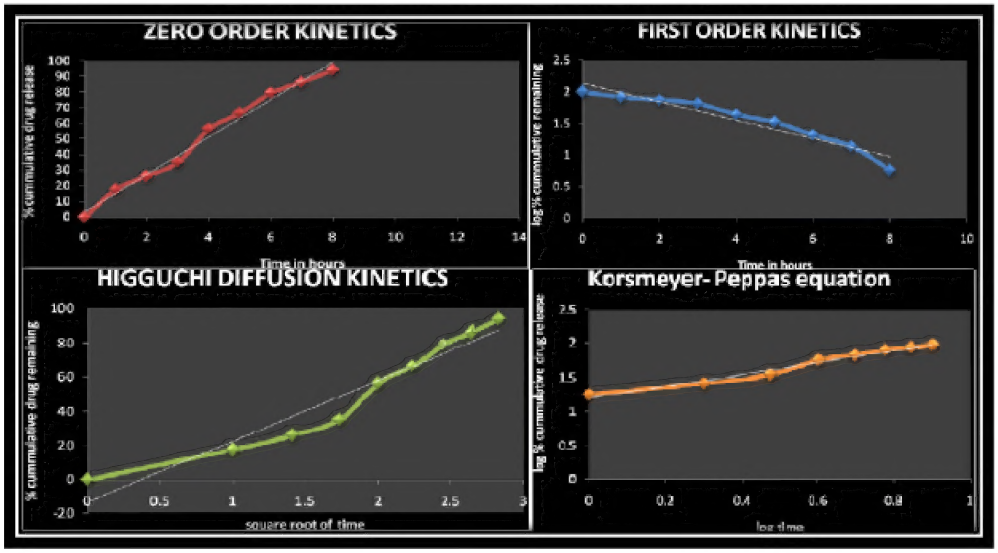
Table 3: Statically ANOVA analysis done in design expert 8.0.4

Variable	Effects	t value	p value	SS
Mean	384.988	106.501	0.000	1333940.250*
A	33.532	-7.574	0.000	6746.236*
B	-40.143	9.067	0.000	9668.922*
a	4.514	1.766	0.069	366.753
b	5.628	-2.202	0.039	570.094*
AB	0.903	0.166	0.437	3.258
Ab	-0.736	-0.235	0.412	6.497
Ba	-0.696	-0.222	0.416	5.810
Ab	-0.256	-0.142	0.446	2.366

Mean, A, B, b are having significant effect on the design; X1,X2, $X2^2$ shows $p<0.001$ is highly significant to the given model. The Quadratic model shows high fitting to the given results for the design. The Study of effects clearly shows that A=Chitosan shows a positive effect, B=PAA shows a negative effect, table no 3 represents the code of A and B.

Figure 4: Release kinetics study of the optimized formulation.



Evaluation of hydrogels

Table 4 indicates that the formulations F1 –F9 prepared with specified amount of polymers to drug. All most all the formulations shows good swelling index figure 6 and the mucoadhesion is optimum for F6 as the time increases the percentage of mucoadhesion gradually decrease figure 7.

Table 4: Study of the cumulative percentage drug release of selected nine formulations

Time (hrs)	*Cumulative percentage drug release*								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	9.317	12.74	14.96	11.51	18.84	17.733	11.083	17.73	16.62
2	18.13	19.46	23.35	19.247	25.597	26.145	21.12	28.36	21.68
3	25.91	27.88	32.9	27.02	36.82	35.157	29.55	38.494	29.011
4	32.08	43	48.6	38.68	51.98	56.41	45.23	56.44	51.89
5	44.86	47.12	64.94	46.02	67.79	66.69	48.8	70.052	59.716
6	61.07	62.34	73.61	72.04	75.921	79.254	66.25	77.64	70.24
7	77.78	72.66	80.17	80.1	79.664	86.339	74.929	83.05	80.6
8	79.34	77.31	84.00	83.33	86.194	94.211	81.43	87.38	89.7

Figure 5: cumulative percentage drug release of the nine formulations

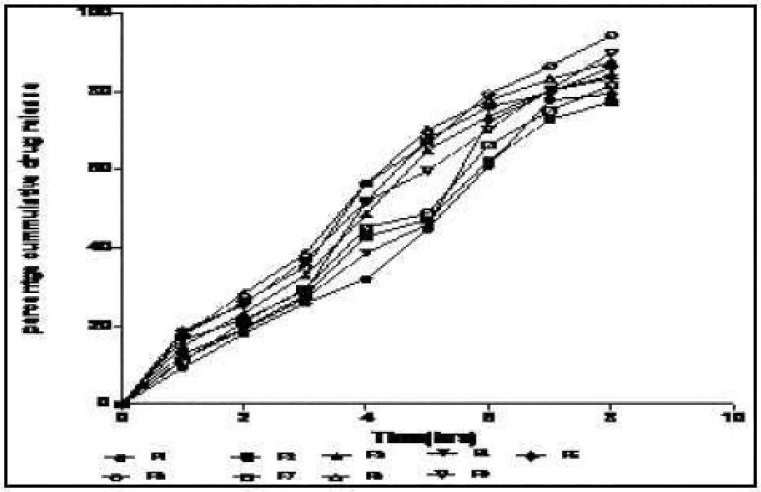


Figure 6: The percentage of swelling gradually increases up to 60-70% Maximum.

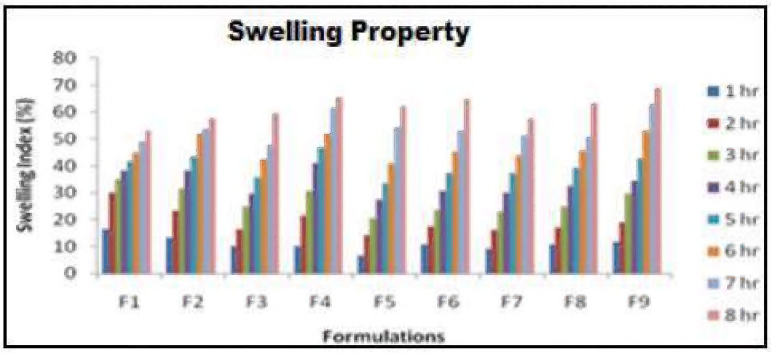
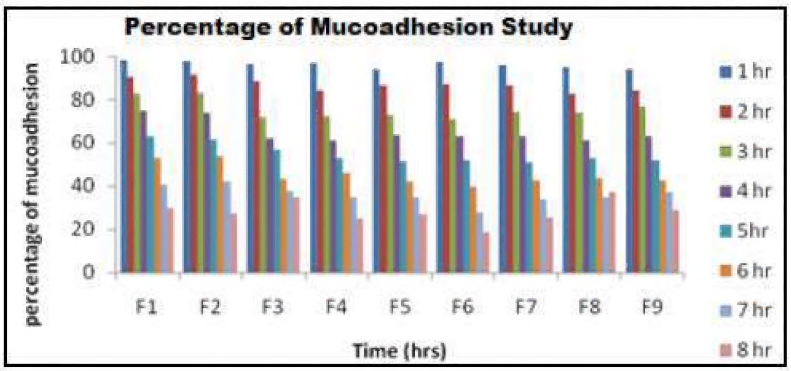


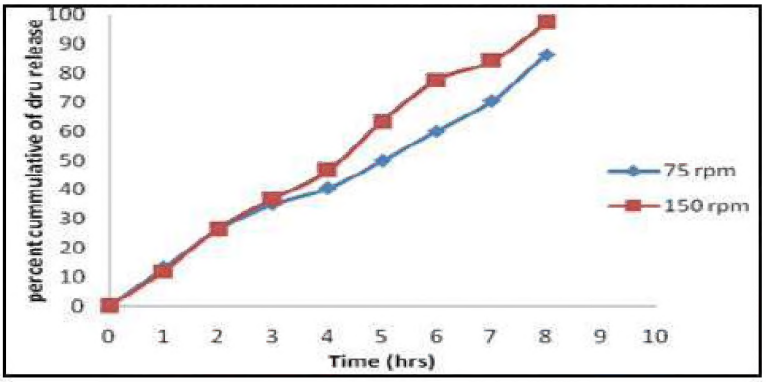
Figure 7: states that percentage of mucoadhesion gradually decreases from 1hr-8hr



Influence of drug release on agitation

To study the effect of agitation intensity on drug release were carried out in dissolution apparatus at various rotation speed,75 rpm,100 rpm,150 rpm) there was no much difference observed in drug release 89.42%,94.21%,97.25% as figure 8. So through the work 100 rpm is kept constant.

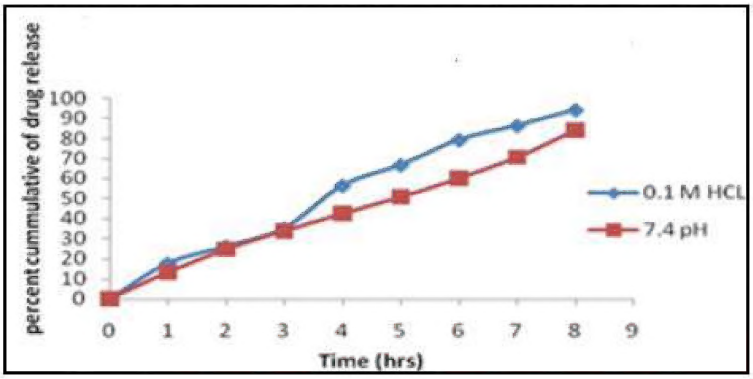
Figure 8: The % cumulative drug release at the end of eight hour for rpm 75 is 86.42%, and for rpm 150 is 97.25 %.



In vitro drug release in different pH 0.1M HCL& 7.4pH

The in vitro drug releases 0.1M HCL when compared with 7.4 pH. 7.4 pH shows less percent of drug release. The percentage drug release is 94.21% and 84.26% given in figure 9.

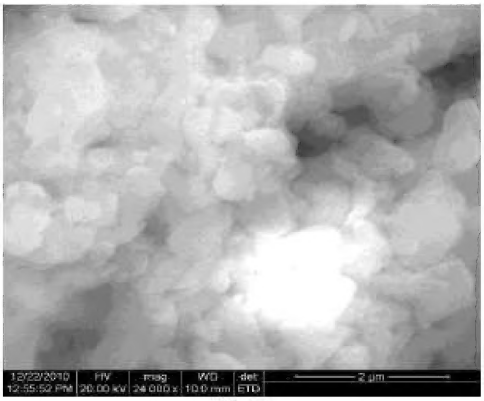
Figure 9: The percent cumulative drug release in 0.1M Hcl & in 7.4 p^H with respect to time



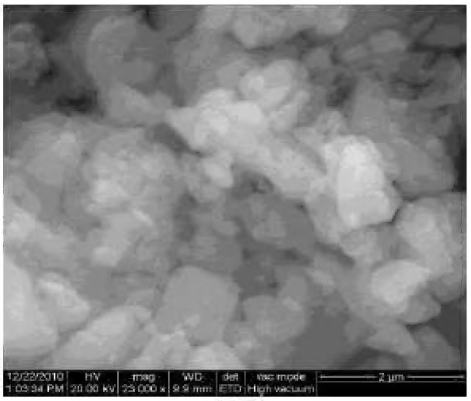
Scanning Electron Micrograph:

The SEM photographs of Figure 10, a, b, c, d hydrogel particles shown surface morphology at different magnification, 24000 x 2□ m , 23000 x 2□ m and 14000 x 2□ m the sponge like fiblilar structure. This is due to the more ionic charge interaction between cationic group of Chitosan and anionic group of polyacrylic acid, the bright area shows that more charge present.

Figure 10 (a, b, c, d): The SEM diagram of various hydrogel particles shown surface morphology at different magnification.



10 (a)



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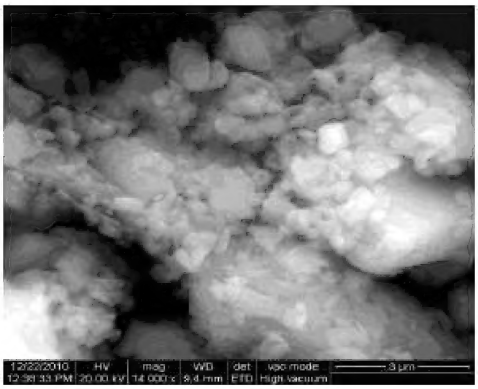

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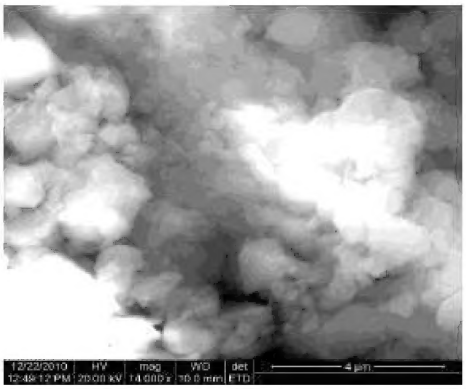

10(d)

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